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A CRITICAL REVIEW ON DRUG STANDARDIZATION

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ABSTRACT

Nowadays, world is witnessing an unprecedented growth in the usage of herbal products. The term "herbal drugs" denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying, and storage. Standardization of drug means confirmation of its identity, quality and purity throughout all phases of its cycle The rise in the use of herbal product has also given rise to various forms of abuse and adulteration of the products leading to consumer's and manufacturer's disappointment and in some instances fatal consequences. For global harmonization WHO specific guidelines for the assessment of the safety, efficacy and quality of herbal medicines are of utmost importance. For standardization and quality assurance purposes, following three attributes are desirable i) Authenticity, ii) Purity and iii) Assay. In order to prove constant composition of herbal preparations, adequate analytical methods have to be applied such as photometric analysis, TLC, HPLC, and GC, metabolomics technique, differential pulse polargraphy chemometric, X-ray diffraction, Capillary electrophoresis, DNA Fingerprinting. Authenticity relates to proving that the material is true. The present overview covers the Standardization parameters with their standards value of the some herbal drugs.

KEYWORDS: *Herbal drugs, Standardization, WHO, Chromatography, Electrophoresis, Herbal medicine, Standardization, DNA finger printing.*

INTRODUCTION

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. There are at least 120 distinct chemical substances derived from plants that are considered as important drugs currently in use in the world, while several other drugs are simple synthetic modifications of the natural products.

Indian healthcare consists of medical pluralism and ayurveda still remains dominant compared to modern medicine, particularly for treatment of a variety of chronic disease conditions.[1] WHO defines traditional medicine as including diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well being, as well as to treat, diagnose or prevent illness. Herbs include crude plant material, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered. Herbal preparations are the basis for finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionation, purification, concentration, or other physical or biological processes. Herbal medicines are used very commonly in various health practices or therapies of Traditional Medicines like Chinese medicine, Ayurveda, Unani, Naturopathy, Osteopathy and Homeopathy.[2]

Standardization in recent years, there has been great demand for plant derived products in developed countries. In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis. American Herbal Product association defines: "Standardization refers to the body of information and control necessary to product material of reasonable consistency. This achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes.[3] "Standardization" expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It also encompasses the entire field of study from birth of a plant to its clinical application. It also means adjusting the herbal drug preparation to a defined content

of a constituent or a respectively by adding excipients or by mixing herbal drugs or herbal drug preparations.[4] "Evaluation" of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration.[5] Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity.[6]

CONCEPT OF STANDARDIZATION

In olden days vaidyas used to treat patients on individual basis, and prepare drug according to the requirement of the patient. In almost all the traditional system of medicine, the quality control aspect has been considered from its inspection of its Rishis, Vaidyas and Hakims. Unlike in olden times where traditional practitioners prepared and tested the qualities of herbal medicines, the problem faced today are these of economics of industrial scale production, shelf life and distribution to long distances. These have necessitated development of modern and objective standards for evaluating the safety, quality and efficacy of these medicines. People are also becoming aware of the potency and side effect. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence standardization is a tool in the quality control process.[7-8] The authentication of herbal drugs and identification of adulterants from genuine medicinal herbs are essential for both pharmaceutical companies aswell as public health and to ensure reproducible quality of herbal medicine.[9]

STANDARDIZATION OF HERBAL FORMULATION

Standardization of herbal formulation requires implementation of Good Manufacturing Practices(GMP). In addition, study of various parameters such as pharmacodynamics, pharmacokinetics, dosage, stability, self-life, toxicity evaluation, chemical profiling of the herbal formulations is considered essential.[10] Other factors such as pesticides residue, aflatoxine content, heavy metals contamination, Good Agricultural Practices (GAP) in herbal drug standardization are equally important.[11]

STANDARDIZATION OF POLYHERBAL FORMULATION

Standardization minimizes batch to batch variation; assure safety, efficacy, quality and acceptability of the polyherbal formulations. The standardization of various marketed herbal and polyherbal formulation Madhumehari Churna (Baidynath) containing the mixture of eight herbal.[12] Dashamularishta, a traditional formulation, used in the normalization of physiological processes after child birth.[13] TLC and HPTLC fingerprint profiles were used for deciding the identity, purityand strength of the polyherbal formulation and also for fixing standards for this Ayurvedic formulation.[14]

WHO GUIDELINES FOR QUALITY STANDARDIZED OF HERBAL DRUGS

1) Quality control of crude drugs material, plant preparations and finished products.

2) Stability assessment and shelf life.

3. Safety assessment; documentation of safety based on experience or toxicological studies.

4) Assessment of efficacy by ethno- medical information and biological activity evaluations.

The bioactive extract should be standardized on the basis of active principles or major compounds along with the chromatographic fingerprints (TLC, HPTLC, HPLC, and GC). Generally, all medicines, whether they are synthetic or of plant origin, should fulfil the basic requirement of being safe and effective.[15-16]

Microscopic and histologic evaluation: These are valuable in both whole as well as powdered drug. It mainly includes study of characteristics like parenchyma, trichomes, calcium oxalate crystals, vascular bundle arrangements, stomata, fibres etc. Qunatitative microscopic study: Microscopic determination such as vein islet number, stomatal index, stomatal number, vein termination number, size of fibres, palisade ratio. Such study helps in differentiation of closely allied species. Physical evaluation: study of various physical parameters like moisture content, solubility, viscosity, refractive index, melting point, optical rotation, ash values, extractives and foreign organic matter.

QUALITY CONTROL OF HERBAL DRUGS

Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. In general, quality control is based on three important pharmacopeial aspects.

a) Identity or authenticity- it should have one herb

b) Purity – it should not have any contaminant other than herb

c) Assay or Content -the active constituents should be within the defined limits. Identity can be achieved by macro and microscopical examinations.

In addition to this identity tests, which include simple chemical tests, eg.colour or precipitation and chromatographic tests are also necessary. Voucher specimens are reliable reference sources. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification[17-18] Purity is closely linked with safe use of drugs and deals with factors such as ash values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation also includes microbial contamination, aflatoxins, radioactivity, and pesticide residues. Analytical methods such as photometric analysis, Thin layer chromatography (TLC), High performance liquid chromatography (HPLC), High performance thin layer chromatography (HPTLC), and Gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations. Content or assay is the most difficult area of quality control to perform, since in most herbal drugs the active constituents are unknown. Sometimes markers can be used. In all other cases, where no active constituents or marker can be defined for the herbal drug, the percentage extractable matter with a solvent may be used as a form of assay, an approach often seen in pharmacopeia.[19-20] A special form of assay is the determination of essential oils by steam distillation. When active constituents (e.g. sennosides in senna) or markers (e.g. alkydamides in Echinacea) are known, a vast array of modern chemical analytical methods such as ultraviolet/visible spectroscopy (UV/VIS), TLC, HPLC, HPTLC, GC, mass spectrometry, or a combination of GC and MS(GC/MS), can be employed.[21] Stability Assessment And Shelf Life Prolonged and apparently uneventful use of a substance usually offers testimony of its safety. In a few instances, however, investigation of the potential toxicity of naturally occurring substances widely used as ingredients in these preparations has revealed previously unsuspected potential for systematic toxicity, carcinogenicity and teratogenicity. Regulatory authorities need to be quickly and reliably informed of these findings. They should also have the authority to respond promptly to such alerts, either by withdrawing or varying the licences of registered products containing suspect substances, or by rescheduling the substances to limit their use to medical prescription.

ASSESEMENT OF QUALITY

All procedures should be in accordance with good manufacturing practices. Crude Plant Material The botanical definition, including genus, species and authority, description, part of the plant, active and characteristics constituents should be specified and, if possible content limits should be defined. Foreign matter, impurities and microbial content should be defined or limited. Voucher specimens, representing each lot of plant material processed, should be authenticated by a qualified botanist and should be stored for at least a 10-year period. A lot number should be assigned and this should appear on the product label.

PLANT PREPARATIONS

The manufacturing procedure should be described in detail. If other substances are added during manufacture in order to adjust the plant preparation to a certain level of active or characteristics constituents or for any other purpose, the added substances should be mentioned in the manufacturing procedures. A method for identification and, where possible, assay of the plant preparation should be added. If identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances to ensure consistent quality of the preparation.

FINISHED PRODUCT

The manufacturing procedure and formula, including the amount of excipients, should be described in detail. A finished product specification should be defined to ensure consistent quality of the product. The finished product should comply with general requirements for particular dosage forms.

SAFETY ASSESSMENT

Herbal medicines are generally regarded as safe based on their long-standing use in various cultures. However, there are case reports of serious adverse events after administration of herbal products. As a whole, herbal medicines can have a risk of adverse effects and drug-drug and drug-food interactions if not properly assessed. Evaluation of the toxic effects of plant constituents of herbal formulation requires detailed phyto-chemical and pharmacological studies. Adulteration of botanical preparations is another important issue. Due to over exploitation of certain plants, habitat loss and fragmentation of the forest, many medicinal plants have reached to the level of the endangered or rare species. The intentional use of pharmaceutical adulterant is possible. Agrochemicals are used to protect the plant from the crude plant material. At the same time growing number of reports about fatal or adverse

effects of herbal preparations intensifies need for national regulation and registration of herbal medicines and establishment of safety monitoring. Clinicians should not prescribe or recommend herbal remedies without well-established efficacy as if they were medications that had been proved effective by rigorous study.[22]

ASSESSMENT OF TOXICITY

Toxicity investigation will also be required because the analysis alone is unlikely to reveal the contributions to toxicity itself. In assessing toxicity of an herbal medicine, the dose chosen is very important.[23] Toxicity assessment involves one or more of the following techniques- In vivo techniques, in vitro techniques, cell line techniques, micro- array and other modern technique, standardization and techniques to adequately model toxicity.

ASSESSMENT OF EFFICACY

Herbal medicines are inherently different from conventional pharmacological treatments, but presently there is no way to assess their efficacy other than by currently used conventional clinical trial methodologies, in which efficacy is conventionally assessed by clinical, laboratory, or diagnostic outcomes: Implementation of a standardized approach for the herbal practitioners and collection of the prospective data necessarily creates an interventional design which, if planned properly, may closely resemble single-blind randomized trials. Even if it differs from double-blind randomized trials in the degree of rigor, the design may be the optimum, both biologically and economically, for rapid evaluation of herbal products. Although randomized clinical trials (with double blind trials as the gold standard) are relatively difficult to be implemented in the case of herbal medicine, they are not ruled out per se in assessing the efficacy of these products. Standardization and Quality control of herbal drugs involve wide array of scientific investigations, which include physical, chemical and biological evaluation employing various analytical method and tools.

A. Physical Evaluation- Each monograph contains detailed botanical, macroscopic and microscopic descriptions with detailed illustrations and photographic images which provide visual documentation of accurately identified material. A microscopic analysis assures the identity of the material and as an initial screening test for impurities.
B. ChemicalEvaluation- Chemical analysis of the drug is done to assess the potency of vegetable material in terms of its active principles. It covers screening, isolation, identification, and purification of the chemical components. It help to determine the identity of the drug substance and possible adulteration.

C. Analytical Methods- It helps in determining identity, quality and relative potency.The basic operation includes steps such as pre- washing, drying of plant materials or freeze-drying and grinding, to obtain a homogenous sample and often improving the kinetics of extraction of the constituents. In the pharmacopoeial monographs, method such as sonication, heating under reflux, Soxhlet extraction, and others are commonly used. However, such methods can be time-consuming, require the use of a large amount of organic solvent, and may have lower extraction efficiencies. New methods are continuously being sought to address this issue. As target compounds may be polar or non polar and even thermally labile, the suitability of the methods of extraction must be considered. To reduce or eliminate the use of organic solvents and improve the extraction processes, newer sample preparation methods, such as microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE) or pressurized liquid extraction (PLE) have been introduced for the extraction of targeted constituents present in plant materials.

CONVECTIONAL METHOD FOR STANDARDIZATION OF HERBAL DRUG FORMULATION

CHROMATOGARPHY

Separation of individual components from the herbal mixture is the key step to enable identification and bioactivity evaluation. Chromatography is a powerful analytical method suitable for the separation and quantitative determination of a considerable number of compounds, even from a complex matrix. These include paper chromatography (PC), thin-layer chromatography (TLC), gas chromatography (GC), HPLC, and capillary electrophoresis (CE). TLC is used extensively in the phytochemical evaluation of herbal drugs because It enables rapid analysis of herbal extracts with minimum sample clean-up requirement, It provides qualitative and semi quantitative information of the resolved compounds.

THIN LAYER CHROMATOGRAPHY (TLC)

TLC is one of the most popular and simple chromatographic technique used of separation of compounds. In the phytochemical evaluation of herbal drugs, TLC is the common fingerprint technique for herbal analysis. The herbal compounds can easily identified by TLC.[41] In this technique, the authentication of various species, evaluation of stability and consistency of their preparations from different manufacturers TLC is being employed extensively for the following reasons.

1. It enables rapid analysis of herbal extracts with minimum sample clean-up requirement,
2. It provides qualitative and semi quantitative information of the resolved compounds.
3. It enables the quantification of chemical constituents.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

HPTLC is the common fingerprint mainly used to analyze the compounds which is having low or moderate polarities. HPTLC technique is widely used in the pharmaceutical industry for process development, identification and detection of adulterants, substituent in the herbal products and also helps in the identification of pesticide content, mycotoxins and in quality control of herb and health products.[24] HPTLC technique was reported for simultaneous estimation of gallic acid, Rutin, Quercetin in terminalia chebula[25] Syzygium Jambolanum was quantitativelyevaluated in terms of stability, repeatability, accuracy and phyto constituents such as glycoside (jamboline),tannin, ellagic acid and gallic acid by HPTLC[26] It was also used for detection, monitoring and quantification of bacoside A & B in Bacopa monnieria and its formulation.[52] Also HPTLC method for phyto constituents in crude drugs or herbal formulations such as bergenin, catechine and gallic acid in Bergenia cilliata and Bergenia lingulata.[27] Chandanasava known to be effective in karsya (malnutrition) was standardised by organoleptic study, physico-chemical analysis, TLC and HPTLC.[28]

GAS CHROMATOGRAPHY

Gas chromatography (GC), also known as gas liquid chromatography (GLC), is a technique for separation of mixtures into components by a process which depends on the redistribution of the components between a stationary phase or support material in the form of a liquid, solid or combination of both and a gaseous mobile phase. It is well-known that many pharmacologically active components in herbal medicines are volatile chemical compounds. Thus, the analysis of volatile compounds by gas chromatography is very important in the analysis of herbal medicines. The GC analysis of the volatile oils has a number of advantages. Firstly, the GC of the volatile oil gives a reasonable “fingerprint” which can be used to identify the plant. The composition and relative concentration of the organic compounds in the volatile oil are characteristic of the particular plant and the presence of impurities in the volatile oil can be readily detected. Secondly, the extraction of the volatile oil is relatively straightforward and can be standardized and the components can be readily identified using GC-MS analysis.[29]

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC is a popular method for the analysis of herbal medicines because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. In general, HPLC can be used to analyze almost all the compounds in the herbal medicines. Thus, over the past decades, HPLC has received the most extensive application in the analysis of herbal medicines. Reversed-phase (RP) columns may be the most popular columns used in the analytical separation of herbal medicines. In order to obtain better separation, some new techniques have been recently developed in research field of liquid chromatography. These are micellar electrokinetic capillary chromatography (MECC), high-speed countercurrent chromatography (HSCCC), low-pressure size-exclusion chromatography (SEC), reversedphase ion-pairing HPLC (RPIPC-HPLC), and strong anion-exchange HPLC (SAX-HPLC). They will provide new opportunities for good separation for some specific extracts of some herbal medicines. On the other hand, the advantages of HPLC lie in its versatility for the analysis of the chemical compounds in herbal medicines, however, the commonly used detector in HPLC, say single wavelength UV detector, seems to be unable to fulfill the task, since lots of chemical compounds in herbal medicines are non-chromophoric compounds. Consequently, a marked increase in the use of HPLC analysis coupled with evaporative light scattering detection (ELSD) in a recent decade demonstrated that ELSD is an excellent detection method for the analysis of non-chromophoric compounds. This new detector provides a possibility for the direct HPLC analysis of many pharmacologically active components in herbal medicines, since the response of ELSD depends only on the size, shape, and number of eluate particles rather than the analysis structure and/or chromophore of analytes as UV detector do.

SUPER CRITICAL FLUID CHROMATOGRAPHY (SFC)

Supercritical fluid chromatography is a hybrid of gas and liquid chromatography that combines some of the best features of each. SFC permits the separation and determination of a group of compounds that are not conveniently handled by either gas or liquid chromatography. SFC has been applied to a wide variety of materials including natural products, drugs, food and pesticide.[30] These compounds are either nonvolatile or thermally labile so that GC procedures are inapplicable or contain no functional group that makes possible detection by the spectroscopic or electrochemical technique employed in LC.[31]

INFRARED SPECTROSCOPY

Near-infrared spectroscopy technique has been used for rapid determination of active components, species, geographic origin, special medicinal formula, on-line quality Control ,identification of counterfeit and discrimination of geographical origins of Chinese herbal medicines. Two-dimensional nearinfrared (NIR) correlation Spectroscopy was applied to the discrimination of Fructus lycii (a traditional Chinese medicinal herb)of four different geographic regions.[32] FTIR along with the statistical method principal component analysis (PCA) was applied to identify and discriminate herbal medicines for quality control in the fingerprint region 400-2000 cm-1. The ratio of the areas of any two marked characteristic peaks was found to be nearly consistent for the same plant from different regions, thereby, an additional discrimination method for herbal medicines. PCA clusters herbal medicines into different groups, clearly showing that IR method can adequately discriminate different herbal medicines using FTIR data.[33]

ELECTROPHORETIC METHODS

Most of the used techniques are capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE) and capillary isoelectric focusing (CIEF). CE is promising for the separation and analysis of active ingredients in herbal medicines, since it needs only small amounts of standards and can analyze samples rapidly with very good separation ability. Also, it is a good tool for producing the chemical fingerprints of the herbal medicines, since it has similar technical characteristics of liquid chromatography.

STANDARDIZATION OF BHASMAS

Bhasmas are made from metals like zinc, lead, gold, silver, tin, copper, metal mixtures and alloys as also from gems, coral and mica and some other minerals, etc. These are formed by calcinations of the parents' substances like minerals, etc. in a rigorous, prescribed manner after it has been appropriately purified and emasculated with herbal juices or minerals.[34] Bhasma are widely recommended for treatment of a variety of chronic ailments and are taken along with milk, butter, honey, or ghee to eliminate the harmful effects of metals and enhancing their biocompatibility in the body.[35] The Ayurvedic Formulary of India lists more than one method of preparation of the bhasmas of some metals while the Drug and Cosmetics Act lists several traditional texts that a vaidya can refer to for the preparation of bhasma.[36] A standardization of process and the end product both is called for Pharmacopoeial standards have been published by the Government of India for a large number of single drugs of plant

origin and the work on classical composite formulations is being carried in several human laboratories. The CCRAS has developed a technique called phase spot test for identification and quality assessment of bhasmas.[37] These have involved Atomic Absorption Spectrophotometry (AAS), flame photometry, Inductively Coupled Plasma Atomic Emission Spectrometry (ICP- AES), X-ray diffraction (XRD) analysis and pHmetry, etc. The AAS uses the property of atoms to absorb certain wavelengths of electromagnetic radiation. The amount of light absorbed enables one a quantitative estimate of absorbing element. The ICP- AES uses plasma (e.g. inductively coupled plasma) to generate excited atoms. These atoms emit electromagnetic radiation at a wavelength that is characteristic of a particular element. From a measure of the intensity of this emission one can quantify the concentration of the element present in a sample. The techniques involving X- ray diffraction analysis reveal information about the crystallographic structure (arrangement of molecules in a crystal) to know whether the particles are crystalline or amorphous and the chemical composition and physical properties of materials. From results on particle size distribution and crystal structure, one can determine how well the bhasma process has proceeded. With such equipment in hand, it should be possible to standardize and freeze the SOPs for bhasma preparations. A ten point"s protocol has been suggested for the standardization of bhasmas and the process of their preparations[38].A standardization programme should also distinguish between bhasmas of metals made of either herbs and non-toxic minerals and those with toxic substances.

NEWER METHODS FOR STANDARDIZATION OF HERBAL DRUGS DNA FINGERPRINTING TECHNIQUE

DNA analysis has been proved as an important tool in herbal drug standardization. This technique is useful for the identification of phytochemically indistinguishable genuine drug from substituted or adulterated drug. This concept of fingerprinting has been increasingly applied in the past few decades to determine the ancestry of plants, animals and other microorganisms. Genotypic characterization of plant species and strains is useful as most plants, though belonging to the same genus and species, may show considerable variation between strains. Additional motivation for using DNA fingerprinting on commercial herbal drugs is the availability of intact genomic DNA from plant samples after they are processed. The other useful application of DNA fingerprinting is the availability of intact genomic. DNA markers are helpful to identity cells, individuals or species as they can be used to produce normal, functioning proteins to replace defective ones. Moreover, these markers help in treatment of various diseases and help in distinguishing the genuine herb from adulterated

drug. Deoxyribonucleic acid (DNA) is the fundamental building component of all living cells. Central Dogma theory can be defined as the fundamental theory of molecular biology that genetic information flows from DNA to RNA to proteins. Inter-Simple Sequence Repeat (ISSR) a PCR-based application is unique and inexpensive popular technique of DNA finger printing which include the characterization of genetic fingerprinting, gene tagging, detection of clonal variation, phylogenetic analysis, detection of genomic instability, and assessment of hybridization.[39-40] Cannabis sativa and Arabidopsis thaliana L. Heyne have been differentiated from their adulterated species by using ISSR markers. DNA based molecular markers have been found to be useful in differentiating different accessions of Taxus wallichiana, Azarchdichta indica, Juniperus communis L., Codonopsis pilosula, Allium schoenoprasum L., Andrographis paniculata collected from different geographical regions. Cannabis sativa and Arabidopsis thaliana L. Heyne have been differentiated from their adulterated species by using ISSR markers.[41]

ROLE OF GENETIC MARKERS IN THE STANDARDIZATION OF HERBAL DRUGS

A genetic marker is a gene or DNA sequence with a known location on a chromosome and associated with a particular gene or trait. It can be described as a variation, which may arise due to mutation or alteration in the genomic loci that can be observed. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism SNP), or a long one, like minisatellites. Some commonly used types of genetic markers are RFLP (or Restriction fragment length polymorphism), AFLP (or Amplified fragment length polymorphism), RAPD (or Random amplification of polymorphic DNA), VNTR (or Variable number tandem repeat), Micro satellite polymorphism- SNP (or Single nucleotide polymorphism), STR (or Short tandem repeat), SFP (or Single feature polymorphism). They can be further categorised. RAPD based molecular markers have been found to be useful in differentiating different accessions of neem collected from different geographical regions.[42] Germplasm analysis to study genetic diversity is another important area in which a lot of efforts have been put in. Fingerprinting of crops like rice wheat, chickpea, pigeon pea, pearlmilletetc is being carried out extensively.[43] Sequence characterized amplified region (SCAR), AP– PCR, RAPD and RFLP have been successfully applied for differentiation of these plants and to detect substitution by other closely related species. e.g. P. ginseng is often substituted by P. quinquefolius (American ginseng).[44]

CHEMOMETRIC METHODS

Chemo metrics is a statistical approach to analyze instrument data, chemometrics often results in a faster and more precise Assessment of composition of a product or even physical or sensory properties. For example, composition (fat, fiber, moisture, carbohydrate) of dairy products or grain can be quickly measured using near infrared spectroscopy and chemometrics. Food properties (e.g., taste, smell, astringency) can also be monitored on a continuous basis. The two general applications of chemometrics technology to predict a property of interest and to classify the sample into one of several categories (e.g., good versus bad, Type A versus Type B versus Type C etc.).Chemometrics is designed to recognize patterns in virtually any type of multidimensional analytical data. Chemometrics can be used to speed methods development and make routine the use of statistical models for data analysis. Keeping in view of the complexity of the chromatographic fingerprint and the irreproducibility of chromatographic and spectral instruments and experimental conditions, several chemometric approaches such as variance analysis, peak alignment, correlation analysis and pattern recognition were employed to deal with the chromatographic fingerprint. The basic principles for this approach are variation determination of common peaks/ regions and similarity comparison with similarity index and linear correlation coefficient. To facilitate the data processing, software named Computer Aided Similarity Evaluation (CASE) has been developed. All programs of chemometric algorithms for CASE are coded in METLAB5.3 based on windows. Data loading, removing, cutting, smoothing, compressing, background and retention time shift correction, normalization, peak identification and spectral matching, variation determination of common peaks/regions, similarity comparison, overly of sample classification and other data processes associated with the chromatographic fingerprint can be investigated with this software.[45-46]

METABOLOMIC TECHNIQUES

Metabolomics is a advanced emerging field of 'omics' research that is concerned with characterizing large numbers of metabolites using NMR, chromatography and mass spectrometry. It is comanly used in biomarker identification and the metabolic profiling of cells, tissues or organisms. The data processing challenges in this technique are quite unique and often require specialized (or expensive) data analysis software. Metabolomics has been used for identification of active phytoconstituents from herbal medicine.[47] Metabolomic approach was employed to identify the chemical constituentsin Sophora flavescens, which were further analyzed for their effect on Pregane X receptor activation and Cytochrome P3A

regulation. The greater potential of metabolomics has been reported in the development of active secondary metabolites from medicinal plants as novel or improved phytotherapeutic agents.[48-49] The recent studies showed that NMRbased metabolomics approach combined with orthogonal projections to latent structurediscriminant analysis identified the purity of an herbal medicine.[50]

DIFFERENTIAL PULSE POLAROGRAPHY (DPP)

DPP can be used to study trace amounts of chemicals with very small detection limits on the order of 10-8 M. Some heavy metals, including lead, cadmium, zinc, copper and iron were successfully identified and determined in chamomile and calendulea flowers by DPP.[51-52] Accumulation of heavy metals, namely Pb, Cd, Cu and Zn was estimated in marketed as well as genuine samples of important herbal drugs of India viz., Alpinia galanga, Artemesia parviflora, Butea monosperrma, Curcuma amada, Euphorbia prostrate, , Malaxis accuminata etc. The concentration of Pb and Cd was found beyond the WHO permissiblelimits in most samples.[53] Trace amounts of selenium in Chinese herbal medicines[54] and flavonoids in small amount of medicinal herb samples were determined byDPP.[55] A DPP method has been for the determination of total hypericin in phytotherapeutic preparations in various buffer systems over the pH range 3.5–10.0.[56]

X-RAY POWDER DIFFRACTROMETRY (XRPD)

This technique is used to identify minerals, crystalline materials and metallic based herbal formulations. The tin based herbal drug Vanga Parpam was estimated by XRD and the intense sharp diffraction peaks clearly confirmed the presence of high crystallinity in Vanga Parpam.[57] XRD analysis of metallic based Indian traditionally medicine Rassindoor indicated the presence of mercury sulphide which is represented by sharp peak.[58] X-ray powder diffractometry data confirmed the formation of phospholipid complex with emodin, naringenin, quercetin, gallic acid.[59-60]

THERMAL ANALYSIS

Thermogravimetric analysis (TGA), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) have been Used to study diffirent physical or chemical changes in various products including herbal drugs and also used to study preformulation or drug excipient compatibility.[106] TGA may be operated under subambient conditions to analyse alcoholic content in various herbal formulations such as asavas and arista.[61] TGA and DTA Analystical method are used to determine mercury based Indian traditional metallic herbal

drug Ras-sindoor indicated the presence of mercury sulphide based on a sharp peak at 354^{o}C which corresponded to melting temperature of mercury sulphide.[62] It is also used in determination of metals present in Bhasma. The optimized extraction obtained by distillation showed the presence of volatile oil in dry ginger as a component of volatile oil-beta-cyclodextrin inclusion compound using DTA technology.[63]

MASS SPECTROSCOPY

Recent advances includes electrospray, thermospray, and ionspray ionization techniques which offer unique advantages of high detection sensitivity and specificity, liquid secondary ion mass spectroscopy, later laser mass spectroscopy with 600 MHz offers accurate determination of molecular weight proteins, peptides. Isotopes pattern can be detected by this technique.[64] LC-MS has become method of choice in many stages of drug development. Chemical standardization of an aqueous extract of the mixture of the 20 herbs provided 20 chemical compounds serving as reference markers using LC-MS.[65] Further, LC-MS analysis of aminoglycosides showed that these drugs are highly soluble in water, exhibited low plasma protein binding, and were more than 90% excreted through the kidney. Further this technique helps in analysis of aminoglycosides in plasma samples with ion pairing chromatography.[66]

NUCLEAR MAGNETIC RESONANCE

The recent introduction of pulsed field gradient technique in high resolution NMR as well as threedimensional technique improves application in structure elucidation and molecular weight information. These new hyphenated techniques are useful in the areas of pharmacokinetics, toxicity studies, drug metabolism and drug discovery process[67]. The combination of chromatographic separation technique with NMR spectroscopy is one of the most powerful and time saving method for the separation and structural elucidation of unknown compound and mixtures, especially for the structure elucidation of light and oxygen sensitive substances.

CONCLUSION

Herbals are traditionally considered harmless and increasingly being consumed by people without prescription. With the tremendous increase in traditional herbal therapy several concerns regarding the safety and quality of herbal medicines have also been observed. Thus,there is need for more advanced techniques of standardization and Quality evaluation of herbal preparation which is a fundamental requirement of industry and other organization dealing with ayurvedic and herbal products. The advancement of analytical techniques will

serve as a rapid and specific tool in the herbal research, thereby, allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities for therapeutic efficacy, safety and shelf- life of herbal drugs. There are basically two techniques used for standardization these are chromatographic fingerprinting and DNA fingerprinting. The chromatographic fingerprinting is based on the chromatographic separation and identification of marker compound from other constituents. For these purpose TLC, HPTLC, HPLC, LC-MS, LC-NMR, GC-MS, GC-FID and SFC methods are used and The other method used is DNA fingerprinting. Quality control of herbal medicines has not only to establish reasonable analytical methods for analyzing the active constituents in herbal medicines, but many other factors should be concerned, such as pesticides residue, aflatoxins content, the heavy metals contamination, good agricultural practice (GAP), good manufacturing practice (GMP), etc. There is need for development of techniques which includes both traditional methods of evaluation and modern methods of evaluation. This will improve the quality of the drug and also motivates the practitioners to get more involved in the standardization process.

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